

FILE 'HOME' ENTERED AT 09:14:57 ON 09 MAR 2009

=> index bioscience  
'BIOSCEINCE' IS NOT A VALID FILE NAME  
ENTER A FILE NAME OR (IGNORE):end

=> index bioscience  
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED  
COST IN U.S. DOLLARS  
FULL ESTIMATED COST

SINCE	FILE	TOTAL
ENTRY		SESSION
	0.22	0.22

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,  
AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,  
CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,  
DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 09:15:30 ON 09 MAR 2009

68 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view  
search error messages that display as 0\* with SET DETAIL OFF.

=> s ferrocen? (s) (prote6 or peptidase or hydrol4 or cleav3 or digest4)  
UNMATCHED RIGHT PARENTHESIS '4'  
The number of right parentheses in a query must be equal to the  
number of left parentheses.

=> s ferrocen? (s) (prote6 or peptidase or hydrol4 or cleav3 or digest4)  
UNMATCHED RIGHT PARENTHESIS '4'  
The number of right parentheses in a query must be equal to the  
number of left parentheses.

=> s ferrocen? (s) (prote##### or peptidase or hydrol### or cleav### or  
digest###)

5	FILE AGRICOLA
71	FILE ANABSTR
3	FILE ANTE
45	FILE BIOENG
56	FILE BIOSIS
65	FILE BIOTECHABS
65	FILE BIOTECHDS
30	FILE BIOTECHNO

13 FILES SEARCHED...

14	FILE CABA
918	FILE CAPLUS
17	FILE CEABA-VTB
1	FILE CONFSCI
4	FILE DDFB
14	FILE DDFU
16	FILE DGENE

23 FILES SEARCHED...

57	FILE DISSABS
4	FILE DRUGB
20	FILE DRUGU
3	FILE EMBAL
84	FILE EMBASE
81	FILE ESBIOBASE
3	FILE FROSTI
5	FILE FSTA

35 FILES SEARCHED...

1	FILE HEALSAFE
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```
116 FILE IFIPAT
43 FILE LIFESCI
85 FILE MEDLINE
19 FILE NTIS
227 FILE PASCAL
47 FILES SEARCHED...
2 FILE PHIN
11 FILE PROMT
288 FILE SCISEARCH
7 FILE SYNTHLINE
51 FILE TOXCENTER
59 FILES SEARCHED...
1020 FILE USPATFULL
32 FILE USPATOLD
230 FILE USPAT2
168 FILE WPIDS
2 FILE WPIFV
168 FILE WPINDEX
```

40 FILES HAVE ONE OR MORE ANSWERS, 68 FILES SEARCHED IN STNINDEX

L1 QUE FERROCEN? (S) (PROTE##### OR PEPTIDASE OR HYDROL### OR CLEAV## OR D  
IGEST###)

=> s L1 (s) (mark## or label## or tag##)

```
1 FILE AGRICOLA
15 FILE ANABSTR
12 FILE BIOENG
8 FILE BIOSIS
17 FILE BIOTECHABS
17 FILE BIOTECHDS
6 FILE BIOTECHNO
```

```
13 FILES SEARCHED...
2 FILE CABA
47 FILE CAPLUS
3 FILE CEABA-VTB
1 FILE DDFU
13 FILE DGENE
23 FILES SEARCHED...
3 FILE DISSABS
3 FILE DRUGU
1 FILE EMBAL
6 FILE EMBASE
10 FILE ESBIOBASE
1 FILE FSTA
```

```
35 FILES SEARCHED...
32 FILE IFIPAT
14 FILE LIFESCI
15 FILE MEDLINE
24 FILE PASCAL
47 FILES SEARCHED...
17 FILE SCISEARCH
8 FILE TOXCENTER
88 FILE USPATFULL
```

```
60 FILES SEARCHED...
7 FILE USPAT2
14 FILE WPIDS
14 FILE WPINDEX
```

28 FILES HAVE ONE OR MORE ANSWERS, 68 FILES SEARCHED IN STNINDEX

L2 QUE L1 (S) (MARK## OR LABEL## OR TAG##)

```
=> s L2 (s) substrat#
      3  FILE ANABSTR
      1  FILE BIOENG
      5  FILE BIOTECHABS
      5  FILE BIOTECHDS
      2  FILE BIOTECHNO
13 FILES SEARCHED...
      2  FILE CAPLUS
      2  FILE DGENE
      1  FILE DISSABS
27 FILES SEARCHED...
      2  FILE ESBIOBASE
      1  FILE FSTA
     13  FILE IFIPAT
37 FILES SEARCHED...
      2  FILE LIFESCI
      2  FILE PASCAL
47 FILES SEARCHED...
     19  FILE USPATFULL
      1  FILE USPAT2
62 FILES SEARCHED...
      5  FILE WPIDS
      5  FILE WPINDEX
```

17 FILES HAVE ONE OR MORE ANSWERS, 68 FILES SEARCHED IN STNINDEX

L3 QUE L2 (S) SUBSTRAT#

```
=> d rank
F1          19  USPATFULL
F2          13  IFIPAT
F3           5  BIOTECHABS
F4           5  BIOTECHDS
F5           5  WPIDS
F6           5  WPINDEX
F7           3  ANABSTR
F8           2  BIOTECHNO
F9           2  CAPLUS
F10          2  DGENE
F11          2  ESBIOBASE
F12          2  LIFESCI
F13          2  PASCAL
F14          1  BIOENG
F15          1  DISSABS
F16          1  FSTA
F17          1  USPAT2
```

=> fil f3-f9, f11, f12  
COST IN U.S. DOLLARS

FULL ESTIMATED COST	SINCE FILE ENTRY	TOTAL SESSION
	10.88	11.10

FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED

FILE 'BIOTECHDS' ENTERED AT 09:25:07 ON 09 MAR 2009  
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FILE 'WPIDS' ENTERED AT 09:25:07 ON 09 MAR 2009

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FILE 'WFINDEX' ACCESS NOT AUTHORIZED

FILE 'ANABSTR' ENTERED AT 09:25:07 ON 09 MAR 2009

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FILE 'BIOTECHNO' ENTERED AT 09:25:07 ON 09 MAR 2009

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FILE 'CAPLUS' ENTERED AT 09:25:07 ON 09 MAR 2009

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FILE 'ESBIOBASE' ENTERED AT 09:25:07 ON 09 MAR 2009

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FILE 'LIFESCI' ENTERED AT 09:25:07 ON 09 MAR 2009

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=> s L3

4 FILES SEARCHED...

L4 21 L3

=> dup rem L4

PROCESSING COMPLETED FOR L4

L5 18 DUP REM L4 (3 DUPLICATES REMOVED)

=> s L5 and py<2004

L6 8 L5 AND PY<2004

=> d L6 ibib abs 1-8

L6 ANSWER 1 OF 8 BIOTECHDS COPYRIGHT 2009 THOMSON REUTERS on STN

ACCESSION NUMBER: 2004-09731 BIOTECHDS <<LOGINID::20090309>>

TITLE: Detecting biomolecules e.g. DNA, for disease diagnosis, comprises applying biochemical probe to substrate with biodetection site including electronic resonator or reacting biomolecule with magnetic tag; DNA biosensor, electrical resonator and DNA probe or RNA probe for nucleic acid detection

AUTHOR: BEUHLER A; SHMAGIN I; LACH L

PATENT ASSIGNEE: BEUHLER A; SHMAGIN I; LACH L

PATENT INFO: US 2003027148 6 Feb 2003

APPLICATION INFO: US 2001-916108 26 Jul 2001

PRIORITY INFO: US 2001-916108 26 Jul 2001; US 2001-916108 26 Jul 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-118833 [12]

AN 2004-09731 BIOTECHDS <<LOGINID::20090309>>

AB DERVENT ABSTRACT:

NOVELTY - Detecting a biomolecule comprises applying biochemical probe to substrate with biodetection site including electronic resonator or reacting biomolecule with magnetic tag and contacting the tagged biomolecule to biodetection site on resonator and detecting the number of biomolecules.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) Detecting a biomolecule comprising: (a) providing a substrate with biodetection site(s) including an electrical resonator; applying

biochemical probe(s) to the biodetection site; (b) interacting the biomolecule with the biochemical probe at the biodetection site; and (c) detecting the biomolecule at the biodetection site; (2) Detecting a biomolecule comprises reacting a biomolecule with a magnetic tag , contacting the tagged biomolecule to a biodetection site on a resonator, coupling the biomolecule to probes in solution and detecting the number of biomolecules; (3) A biosensor for assaying biomolecules, comprising: (a) a substrate having at least one biodetection site; and (b) an electrical resonator proximate the biodetection site to allow measurement of magnetic properties at the biodetection site; and (4) A kit for assaying target biomolecules comprising: (a) a sample plate with biodetection sites; (b) a holding gel adapted to coat the biodetection sites; and (c) an electrical resonator operatively attached to the sample plate to allow measurement of magnetic properties of the biodetection sites.

**BIO TECHNOLOGY - Preferred Method:** The method further comprises applying a magnetic tag to the biomolecule and detecting magnetic properties of the biodetection site with the resonator. The value of the magnetic properties of the biodetection site corresponds to a number of biomolecules at the biodetection site. The method further comprises applying a holding substance to the biodetection site. The substrate comprises an organic substrate with embedded resonance frequency structures. The biochemical probe is an oligonucleotide. The magnetic tag is ferrocene. The magnetic properties of the biodetection site are measured before applying the biomolecule. The method further comprises determining an alteration in magnetic properties of the biodetection site. The value of the alteration in magnetic properties with a number of biomolecules is correlated and the number of biomolecules at the biodetection site is determined based upon this value of alteration. **Preferred Biosensor:** The biodetection site includes a holding substance. The biodetection site is adapted to receive biomolecule(s) and further comprises magnetic tag(s) operatively adapted to be applied to the biomolecule. The electrical resonator measures a value of magnetic properties, the value corresponding to a number of biomolecules at the biodetection site. The electrical resonator has a spiral geometry. It further comprises a measuring component for measuring the magnetic properties in communication with the biodetection site and a quantitating element for correlating an amount of biomolecules at the biodetection site to the magnetic properties of the biodetection site. **Preferred Kit:** The kit comprises a solution of biochemical probe(s) adapted to be applied to the biodetection sites and a solution of magnetic tag(s) adapted to be applied to the target biomolecules.

**USE -** The methods and kits are useful for detecting biomolecules such as DNA and RNA.

**EXAMPLE -** A substrate containing multiple surface resonator test sites with fully defined geometry and specifications was fabricated by using standard printed circuit board print and etch technology. A polymer hydrogel coating containing biomolecular probe linker groups was draw coated onto the surface of 10 of the test sites to a thickness of 5 microns. The polymer coating was exposed to UV radiation of 1000 mJ/cm<sup>2</sup> at 365 nm to crosslink the polymer and render it insoluble to water solutions. 40 microl of a 100 microgram/ml streptavidin protein was then washed over the surface of 5 of the resonators. The resonators were irradiated again with UV light at 365 nm (100 mJ/cm<sup>2</sup>) to photoactively couple the streptavidin to the probe linker groups in the polymer coating. The resonators were washed to remove excess material. The resulting protein concentration on the surface was approximately 0.67 nmoles per test site. The resonance frequency of the 5 test sites with the bound streptavidin were compared to 5 test sites with no streptavidin. The test sites containing streptavidin showed

a 14 MHz frequency shift compared to the test sites with no streptavidin. This indicates that 0.67 nmoles per test site of a large protein such as streptavidin can be detected. Each test site is 1.94 cm<sup>2</sup> so this is a sensitivity of 0.35 nmoles/cm<sup>2</sup>. In this case the frequency shift is due to the change in the capacitive properties of the test site due to the presence of streptavidin since the streptavidin does not contain a magnetic tag.(12 pages)

L6 ANSWER 2 OF 8 BIOTECHDS COPYRIGHT 2009 THOMSON REUTERS on STN  
ACCESSION NUMBER: 2003-12443 BIOTECHDS <>LOGINID::20090309>>  
TITLE: Method for detecting ligand-ligate interaction, useful e.g.  
in immunological or hybridization assays, based on  
displacement of labeled signal ligand;  
ligand-ligate interaction and biochip useful for  
hybridization assay  
AUTHOR: HARTWICH G; FRISCHMANN P; HAKER U; WIEDER H  
PATENT ASSIGNEE: FRIZ BIOCHEM GMBH  
PATENT INFO: WO 2003019194 6 Mar 2003  
APPLICATION INFO: WO 2002-DE1269 6 Apr 2002  
PRIORITY INFO: DE 2001-1041691 25 Aug 2001; DE 2001-1041691 25 Aug 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
OTHER SOURCE: WPI: 2003-290094 [28]  
AN 2003-12443 BIOTECHDS <>LOGINID::20090309>>  
AB DERTWENT ABSTRACT:  
NOVELTY - Method for detecting ligate-ligand interactions by contacting a surface, modified by attachment of at least one ligate (L1), with a known amount of signal ligand (SL) and a sample containing a ligand (L2). SL are detected and the result compared with a reference value.  
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a kit for the new method.  
USE - The method is used to detect e.g. antibody/antigen; nucleic acid/nucleic acid or enzyme/substrate, or many other sorts of interactions, particularly with low-density protein/DNA chips for point-of-care systems.  
ADVANTAGE - The method is a simple, inexpensive and reliable displacement assay that does not require modification of the target by labeling.  
EXAMPLE - An oligonucleotide was modified with the residue HO-(CH<sub>2</sub>)-S-(CH<sub>2</sub>)<sub>2</sub>- and immobilized on gold. A complementary nucleic acid tetramer, labeled twice with ferrocene, (10 microM) was applied and chronocoulometric measurements (1) made. The complementary target (5 microM) was added and the measurements (2) repeated. The plots of charge against square root of time are presented and are clearly different between (1) and (2), with the integrated difference between the two curves being 0.7 microC.(57 pages)

L6 ANSWER 3 OF 8 BIOTECHDS COPYRIGHT 2009 THOMSON REUTERS on STN  
ACCESSION NUMBER: 2002-10819 BIOTECHDS <>LOGINID::20090309>>  
TITLE: Interaction of species (e.g. biological ligand) immobilized  
on colloidal particles, with species (e.g. molecule capable  
of reacting with ligand) immobilized on non-colloidal  
structure e.g. magnetic beads, useful in e.g. drug screening;  
biological ligand interaction, colloid particle  
immobilization and magnetic bead, for drug screening  
AUTHOR: BAMDAD C C; BAMDAD R S  
PATENT ASSIGNEE: MINERVA BIOTECHNOLOGIES CORP  
PATENT INFO: WO 2002001228 3 Jan 2002  
APPLICATION INFO: WO 2000-US20168 23 Jun 2000  
PRIORITY INFO: US 2000-602778 23 Jun 2000  
DOCUMENT TYPE: Patent

LANGUAGE: English  
OTHER SOURCE: WPI: 2002-205935 [26]  
AN 2002-10819 BIOTECHDS <>LOGINID::20090309>>  
AB DERWENT ABSTRACT:

NOVELTY - (1) A method comprises allowing a colloid particle the ability to become immobilized with respect to a non-colloidal structure (II); and determining immobilization of the colloid particle relative to (II).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (2) signaling a single binding of a first biological or chemical agent to a second biological or chemical agent with a plurality of signaling entities; (3) determining protein/ligand interaction in the absence of SPR without labeling either the protein or the ligand; (4) a method, comprising: (a) providing: (i) a solution comprising colloids, the colloids comprising a ligand capable of interacting with a cell surface molecule and (ii) a composition comprising an electrode comprising growing cells, the cells comprising at least one cell surface molecule capable of interacting with the ligand; (b) adding at least a portion of the colloids to the composition; (5) a method comprising: (a1) providing: (i) a solution comprising colloids, the colloids comprising a ligand capable of interacting with a cell surface molecule, (ii) a candidate drug, and (iii) a composition comprising an electrode comprising growing cells, the cells comprising at least one cell surface molecule capable of interacting with the ligand; and (b1) mixing at least a portion of the colloids with the drug and the composition; (6) recruiting an electronic signaling entity to an electrode using a magnetic material; (7) an article defining a surface, and a ligand suspected of interacting with a protein and an electroactive entity each immobilized relative to the surface; (8) an article comprising: a first biological or chemical agent, capable of biological or chemical binding to a second agent, immobilized relative to a plurality of signaling entities; (9) an article defining a surface, and a self-assembled monolayer formed on the surface of the article; (10) a composition comprising a first molecule and one or more signaling entities attached to a solid support, where the first molecule is a ligand capable of interacting with a cell-surface receptor or protein; (11) a composition, comprising a first molecule, a second molecule and a third molecule attached to a solid support, where the first molecule comprises a ligand capable of interacting with a cell-surface receptor or protein, where the second molecule forms a monolayer on the solid support, and where the third molecule is electroactive; (12) an article comprising a metal support constructed and arranged to support the growth of cells on its surface, the metal support comprising a monolayer of at least one type of molecule, the monolayer configured such that the metal support can be used as an electrode; (13) a composition comprising: a colloid particle; a signaling entity immobilized relative to the colloid particle; and a protein immobilized relative to the colloid particle; (14) a species comprising: a polymer or dendrimer carrying a plurality of signaling entities adapted for linkage to a biological or chemical agent (I); (15) an article comprising a colloid particle immobilized relative to a glutathione derivative and at least one signaling entity; (16) an article comprising a colloid particle carrying on its surface, a self- assembled monolayer comprising a glutathione derivative.

USE - Methods, compositions, species and articles for detecting or monitoring interactions between chemical and biochemical species, including drug screening assays, are provided. For detecting interactions between ligands and target receptors on the surface of live intact cells to enable screening of candidate compounds which disrupt these interactions. For screening compound libraries for drugs that inhibit the activity of cell surface receptors. For examining e.g. protein/protein, protein/peptide, antibody/antigen,

antibody/hapten, enzyme/substrate, enzyme/inhibitor, enzyme/cofactor, binding protein/substrate, carrier protein/substrate, lectin/carbohydrate, receptor/hormone, receptor/effectector, complementary strands of nucleic acid, protein/nucleic acid, repressor/inducer, ligand/cell surface receptor and virus/ligand interactions. For identifying cell-derived molecules (e.g. receptors or proteins), that are expressed differentially in healthy versus diseased tissue or cells, i.e. diagnostic assays for determination of diseased states. For visually investigating patterns of cell surface receptor expression on individual cell surfaces and/or on cells embedded in a tissue specimen. Biospecific colloids (gold colloids) may be used to facilitate *in vivo* imaging, e.g. detection of tumor using X-ray or X-ray computer tomography.

**ADVANTAGE** - A series of components and techniques for drug screening are provided. The approach provides: (I) modular system for the attachment of natural ligands to universal signaling elements; (ii) enhanced sensitivity of detection through the attachment of a plurality of signaling elements to each ligand; (iii) a simpler format (without the need for washing steps, enzymatic cleavage and toxic substrates); (iv) a convenient electronic output; and (v) the capability of multiplexing. Further advantages over existing methods such as ELISA, fluorescent labeling and SPR include: in the above systems, there is no need for protein labeling; the protein is attached to a labeled component. Gold colloids can be pre-labeled with both: (a3) a signaling moiety; and (b3) a functional group for protein attachment. Self assembled monolayers that present both NTA/Ni<sup>2+</sup>, to capture histidine-tagged proteins, and a ferrocene derivative, for electronic or electrochemical signaling, can be formed on the colloids. SAMs that incorporate carboxylic acid groups, for the chemical coupling (standard EDC/NHS chemistry) of unmodified proteins, can also be used. Virtually any biological species can be co-immobilized on colloids with a signaling entity. The technology enables cost-effective multiplexing as it can readily be multiplexed on microelectrode arrays. (NTA = nitrilo triacetic acid; SAM = self-assembled monolayer).

**EXAMPLE** - A target protein, Glutathione-S-Transferase (GST) was histidine-tagged and immobilized on SAM-coated colloids that presented NTA-Ni (histidine tags bind NTA-Ni). 30 microl of colloids presenting 40 microM NTA-Ni on the surface were added to 65 microl of 21.5 microM GST, to give a final concentration of 14 microM GST in solution. Glutathione, a small molecule that binds GST, is commercially available bound to agarose beads through Sigma-Aldrich. Glutathione-coated beads were incubated with the solution of GST-bound colloids. Within minutes, the GST bound to the glutathione beads, bringing the colored colloids out of solution, and decorating the beads red (Figure 20). Beads displaying a small molecule that does not bind to GST remained colorless when incubated with the GST-bound colloids (Figure 21). A second negative control, in which glutathione-coated beads were incubated with 30 microl NTA-Ni colloids in the absence of GST showed that NTA-Ni-colloids do not bind nonspecifically to the bead surfaces or to the glutathione. (83 pages)

and D-glucose; 20mM-K2HPO4 (pH 6.9) is used as supporting electrolyte. The catalytic current produced (measured by using a Pt-gauze counter-electrode and a SCE) is related rectilinearly to the I concentration (0.4 to 5 $\mu$ M) if the enzyme reactions are not substrate-limited. The magnitude of response is time-dependent.

L6 ANSWER 5 OF 8 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN  
ACCESSION NUMBER: 2000:32002327 BIOTECHNO <>LOGINID::20090309>>  
TITLE: Immunoassay of the MRSA-related toxic protein,  
leukocidin, with scanning electrochemical microscopy  
AUTHOR: Kasai S.; Yokota A.; Zhou H.; Nishizawa M.; Niwa K.;  
Onouchi T.; Matsue T.  
CORPORATE SOURCE: T. Matsue, Dept. of Biomolecular Engineering, Graduate  
School of Engineering, Tohoku University, Sendai  
980-8579, Japan.

SOURCE: Analytical Chemistry, (01 DEC 2000), 72/23  
(5761-5765), 36 reference(s)  
CODEN: ANCHAM ISSN: 0003-2700

DOCUMENT TYPE: Journal; Article  
COUNTRY: United States  
LANGUAGE: English

SUMMARY LANGUAGE: English

AN 2000:32002327 BIOTECHNO <>LOGINID::20090309>>

AB Scanning electrochemical microscopy (SECM) was applied to the immunoassay of leukocidin, which is a toxic protein produced by methicillin-resistant *Staphylococcus aureus* (MRSA), with the intention of developing and early diagnostic for MRSA infection. An antibody - chip for leukocidin was prepared by self-assembling of anti-leukocidin on a protein A-coated glass substrate. A sample solution containing leukocidin was spotted onto the antibody-chip, followed by labeling with horseradish peroxidase (HRP) via a sandwich method. The reduction current of the oxidized form of ferrocenylmethanol generated by the HRP reaction was monitored to view SECM images of the spot of captured leukocidin. The amplitude of reduction current depended on the concentrations of sample solutions used for making spots. This SECM-based immunoassay detects as low as 5.25 pg mL<sup>-1</sup>.

L6 ANSWER 6 OF 8 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN  
ACCESSION NUMBER: 1996:27037958 BIOTECHNO <>LOGINID::20090309>>

TITLE: Enzyme immunoassay with amperometric flow-injection analysis using horseradish peroxidase as a label.  
Application to the determination of polychlorinated biphenyls

AUTHOR: Del Carlo M.; Mascini M.

CORPORATE SOURCE: M. Mascini, Dipartimento Sanita Pubblica,  
Epidemiol/Chimica Analit Ambientale, Universita degli  
Studi di Firenze, Via G. Capponi 9, 50121 Firenze,  
Italy.

E-mail: mascini@cesisti.unifi.it.

SOURCE: Analytica Chimica Acta, (1996), 336/1-3  
(167-174), 25 reference(s)

CODEN: ACACAM ISSN: 0003-2670

PUBLISHER ITEM IDENT.: S0003267096003777

DOCUMENT TYPE: Journal; Article

COUNTRY: Netherlands

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 1996:27037958 BIOTECHNO <>LOGINID::20090309>>

AB An amperometric detection system for horseradish peroxidase (HRP) activity was optimized using flow injection analysis (FIA) with glassy

carbon as a working electrode. Ferroceneacetic acid was investigated as a co-substrate for the electrochemical detection of HRP. The calculated detection limit for HRP was  $2.6 \times 10.0 \text{ sup.-sup.1.sup.2 M}$  with incubation of 30 min. The substrate was used in an electrochemical enzyme immunoassay for polychlorinated biphenyls (PCB). We used a competitive assay, where PCB-protein conjugate (gelatin) was immobilized to the solid phase (microtitre assay plate) and the competition was carried out with PCB standards using a limiting amount of anti-PCB IgG. The extent of the competition was evaluated using a secondary, HRP labelled, IgG; the amount of the enzyme label was detected after 30 min of incubation with the substrate. The PCB range was  $0.1\text{-}50 \mu\text{g ml.sup.-sup.1}$ . The overall assay time was 2 h and 30 min. The within assay precision, over 6 measurements, was lower than 10% for the entire range.

L6 ANSWER 7 OF 8 Elsevier Biobase COPYRIGHT 2009 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1998022420 ESBIOBASE <>LOGINID::20090309>>  
TITLE: Covalent attachment of an electroactive sulphydryl reagent to the active site of cytochrome P450(cam) as revealed by the crystal structure of the modified protein  
AUTHOR(S): Di Gleria, Katalin; Nickerson, Darren P.; Hill, H. Allen O.; Wong, Luet-Lok; Fulop, Vilmos  
CORPORATE SOURCE: Di Gleria, Katalin; Nickerson, Darren P.; Hill, H. Allen O.; Wong, Luet-Lok (Department of Chemistry, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR (GB)); Fulop, Vilmos (Laboratory of Molecular Biophysics, Oxford Centre for Molecular Sciences, University of Oxford, South Parks Road, Oxford OX1 3QU (GB))  
SOURCE: Journal of the American Chemical Society (14 Jan 1998) Volume 120, Number 1, pp. 46-52, 31 refs.  
CODEN: JACSAT ISSN: 0002-7863  
DOI: 10.1021/ja972473k  
COUNTRY OF PUBLICATION: United States of America  
DOCUMENT TYPE: Journal; Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 31 Jan 2009  
Last updated on STN: 31 Jan 2009  
AN 1998022420 ESBIOBASE <>LOGINID::20090309>>  
AB A novel electroactive sulphydryl-specific reagent, N-(2-ferrocenylethyl)maleimide (Fc-Mi), was used to attach a redox-active reporter group to cytochrome P450(cam) from *Pseudomonas putida*. The crystal structure of the modified enzyme was determined at 2.2 Å resolution ( $R(\text{cryst}) = 0.18$ ) and compared to the structure of the wild-type enzyme complexed with its natural substrate. The results showed that two molecules of the electroactive modifier were attached to the protein. One of the ferrocenes was linked to Cys85 via the maleimide moiety and occupied the camphor-binding site in the substrate pocket. The other ferrocene was linked to Cys136 on the surface of the protein. Significant conformational changes were observed on the distal side of the heme when camphor was replaced by ferrocene. The shift in the Soret band from 392 to 417 nm upon modification arose from the binding of a water molecule to the heme iron immediately below the ferrocene in the active site of the modified enzyme. The electrochemistry of the labeled enzyme showed clear signals originating both from the heme and from the covalently linked ferrocenes. The direct current cyclic

voltammogram revealed a striking positive shift in the heme redox potential of the ferrocene-containing P450(cam) from -380 mV for the camphor-bound wild-type protein to -280 mV for the modified protein.

L6 ANSWER 8 OF 8 LIFESCI COPYRIGHT 2009 CSA on STN  
ACCESSION NUMBER: 2000:72742 LIFESCI <>LOGINID::20090309>>  
TITLE: Covalent Attachment of an Electroactive Sulfydryl Reagent  
in the Active Site of Cytochrome P450 sub(cam) as Revealed  
by the Crystal Structure of the Modified Protein  
AUTHOR: Di Gleria, K.; Nickerson, D.P.; Hill, H.A.O.; Wong, L.-L.;  
Fueiop, V.  
CORPORATE SOURCE: Department of Chemistry, Inorganic Chemistry Laboratory,  
University of Oxford, South Parks Road, Oxford OX1 3QR, UK  
SOURCE: Journal of the American Chemical Society [J. Am. Chem.  
Soc.], (19980100) vol. 120, no. 1, pp. 46-52.  
ISSN: 0002-7863.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: J  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB A novel electroactive sulfydryl-specific reagent, N-(2-ferrocenylsethyl)maleimide (Fc-Mi), was used to attach a redox-active reporter group to cytochrome P450 sub(cam) from *Pseudomonas putida*. The crystal structure of the modified enzyme was determined at 2.2 Angstrom resolution ( $R_{\text{sub(cryst)}} = 0.18$ ) and compared to the structure of the wild-type enzyme complexed with its natural substrate. The results showed that two molecules of the electroactive modifier were attached to the protein. One of the ferrocenes was linked to Cys85 via the maleimide moiety and occupied the camphor-binding site in the substrate pocket. The other ferrocene was linked to Cys136 on the surface of the protein. Significant conformational changes were observed on the distal side of the heme when camphor was replaced by ferrocene. The shift in the Soret band from 392 to 417 nm upon modification arose from the binding of a water molecule to the heme iron immediately below the ferrocene in the active site of the modified enzyme. The electrochemistry of the labeled enzyme showed clear signals originating both from the heme and from the covalently linked ferrocenes. The direct current cyclic voltammogram revealed a striking positive shift in the heme redox potential of the ferrocene-containing P450 sub(cam) from -380 mV for the camphor-bound wild-type protein to -280 mV for the modified protein.

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